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REVIEW ARTICLE

Targeting multiple opioid receptors – improved analgesics with reduced side effects?

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Classical opioid analgesics, including morphine, mediate all of their desired and undesired effects by specific activation of the μ-opioid receptor (μ receptor). The use of morphine for treating chronic pain, however, is limited by the development of constipation, respiratory depression, tolerance and dependence. Analgesic effects can also be mediated through other members of the opioid receptor family such as the κ-opioid receptor (κ receptor), δ-opioid receptor (δ receptor) and the nociceptin/orphanin FQ peptide receptor (NOP receptor). Currently, a new generation of opioid analgesics is being developed that can simultaneously bind with high affinity to multiple opioid receptors. With this new action profile, it is hoped that additional analgesic effects and fewer side effects can be achieved. Recent research is mainly focused on the development of bifunctional μ/NOP receptor agonists, which has already led to novel lead structures such as the spiroindole-based cebranopadol and a compound class with a piperidin-4-yl-1,3-dihydroindol-2-one backbone (SR16835/AT-202 and SR14150/AT-200). In addition, the ornivol BU08028 is an analogue of the clinically well-established buprenorphine. Moreover, the morphinan-based nalfurafine exerts its effect with a dominant κ receptor-component and is therefore utilized in the treatment of pruritus. The very potent dihydroetorphine is a true multi-receptor opioid ligand in that it binds to μ, κ and δ receptors. The main focus of this review is to assess the paradigm of opioid ligands targeting multiple receptors with a single chemical entity. We reflect on this rationale by discussing the biological actions of particular multi-opioid receptor ligands, but not on their medicinal chemistry and design.

LINKED ARTICLES

This article is part of a themed section on Emerging Areas of Opioid Pharmacology. To view the other articles in this section visit http://onlinelibrary.wiley.com/doi/10.1111/bph.v175.14/issuetoc

Abbreviations

DRG, dorsal root ganglion; N/OFQ, nociceptin/orphanin FQ peptide; NOP receptor, nociceptin/orphanin FQ peptide receptor; PAG, periaqueductal grey; RVM, rostral ventral medulla

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Introduction

Opioids binding to the μ-opioid receptor (**μ receptor**) remain the mainstay in the management of moderate to severe pain conditions (Smith and Peppin, 2014). However, their long-term use is limited by various side effects, such as constipation, potentially life-threatening respiratory depression, nausea, development of tolerance and physical dependence (Bailey and Connor, 2005). This situation has led to different approaches to develop drugs with a more favourable side effect profile. However, as yet, there is no single compound known in the vast repertoire of orthosteric μ receptor-selective ligands that has analgesic action that is not also associated with side effects. In μ receptor knock out mice, both the antinociception and side effects of **morphine** are clearly mediated by μ receptor (Kieffer and Gaveriaux-Ruff, 2002).

The design and clinical development of new analgesics has to consider several intracacies: (i) the multiplicity of neurochemical transmitters and receptors involved in pain pathways; (ii) the variety of physiological functions mediated by a single transmitter or receptor target; (iii) the different intrinsic activity (*e.g.* full vs. partial agonist vs. antagonist) or mechanism (orthosteric vs. allosteric) of a drug compound; (iv) different signalling mechanisms mediated by a single receptor species (e.g. 'biased' intracellular coupling); (v) receptor oligo- and/or heteromerization; (vi) anatomy, functional hierarchy and redundancy of pain pathways (e.g. central vs. spinal vs. peripheral; nociceptive vs. antinociceptive); (vii) species differences in translational animal models and clinical efficacy in humans; (viii) limited solubility due to high lipophilicity; and (ix) low bioavailability. Obviously, these complexities will multiply when more than a single drug target is therapeutically addressed at the same time. Despite these challenges, many recent efforts are trying to harness the powerful analgesic effects of μ receptor agonists with new strategies to circumvent typical μ receptor agonist-associated side effects and thus demonstrate the insistent need for improved μ receptor-directed analgesics. As one possible strategy to overcome this dilemma, recent studies have proposed the development of so-called 'biased' μ receptor agonists, which are able to induce receptor conformations that preferentially trigger G_i-dependent signalling without activating β-arrestin pathways (Manglik *et al.*, 2016). It is hypothesized that compounds promoting G_i-signalling will produce analgesia while avoiding β-arrestin-dependent effects on respiration or reinforcement. Despite promising preclinical and early clinical data (DeWire *et al.*, 2013; Viscusi *et al.*, 2016), it is currently too early to validate this concept as, so far, only two biased μ receptor agonists have been investigated more closely.

While the μ receptor is the predominant target of morphine-like compounds, analgesia can be mediated by activation of any of the four members of the opioid receptor family, as has been shown in human and non-human settings for the κ-opioid receptor (**κ receptor**) (Pande *et al.*, 1996; Schepers *et al.*, 2008), δ-opioid receptor (**δ receptor**) (Petrillo *et al.*, 2003) and nociceptin/orphanin FQ peptide receptor (**NOP receptor**) (Xu *et al.*, 1996; Yamamoto *et al.*, 1997). However, thus far, the clinical development of selective δ or κ receptor compounds has failed due to limited analgesic potency and/or dysphoric effects in rodents (Eguchi, 2004; Bodnar, 2010) respectively. Although selective κ and δ receptor agonists lack the typical μ receptor-mediated side effects, such as respiratory depression and constipation, they possess their own distinct profile of side effects, including dysphoria, sedation, diuresis and constipation for κ receptor agonists (Land *et al.*, 2008; White *et al.*, 2015), and convulsive effects have been observed with many δ receptor agonists (Jutkiewicz *et al.*, 2006). Activation of the δ receptor has been proved to be effective in chronic pain conditions, a state that can only be insufficiently controlled by μ receptor agonists (Nadal *et al.*, 2006; Gaveriaux-Ruff *et al.*, 2008). As an additional benefit, and in contrast to μ receptor agonists, δ receptor activation causes no physical dependence (Brandt *et al.*, 2001). Furthermore, activation of δ receptors has low abuse liability, as δ receptor agonists are not self-administered (Negus *et al.*, 2009). Other important features of δ receptor agonists are their anxiolytic and anti-depressant effects that are desirable in chronic pain conditions (Perrine *et al.*, 2006), as indicated in Table 1.

Analgesic effects mediated by the NOP receptor are the most complex of any other member of the opioid receptor family. Depending on the exposure to exogenous opioid agonists, **nociceptin/orphanin FQ (N/OFQ)** either blocks the analgesic effect of opioids, or mediates an analgesic effect by reducing hyperalgesia during opioid withdrawal conditions (Pan *et al.*, 2000). Overall, N/OFQ appears to act as a 'functional opioid antagonist' in the CNS. Additionally, the N/OFQ system has different effects on spinal compared to supraspinal nociceptive circuits (Heinricher, 2005), and these pain circuits may differ between rodents and non-human primates (Ko *et al.*, 2009b). Due to the similarities and interactions with the classical opioid peptides and their receptors, the N/OFQ-NOP receptor system was studied early on as a potential drug target on its own and in combination with opioid analgesics. For instance, the joint application of the NOP receptor-selective agonist **Ro64-6198** and morphine in subthreshold analgesic doses reduced pain sensitivity in the hot plate assay in an additive manner (Reiss *et al.*, 2008), although it showed hyperalgesic effects in the tail flick assay when administered alone. Interestingly, Ro64-6198 inhibits pain in rhesus monkeys without mediating opioid-like side effects (Ko and Naughton, 2009a) and displays clinically desirable antiallodynic actions in rats (Obara *et al.*, 2005). However, Ro64-6198 never reached clinical trials, most likely due to low oral bioavailability and substance-specific side effects such as impairment of motor activity, learning and memory (Shoblock, 2007). Other selective NOP receptor agonists like MCOPPB and **SCH221510** probably do not pass through the blood brain barrier, although some studies report anxiolytic effects of MCOPPB, which is indicative of central activity (Hirao *et al.*, 2008). The role of N/OFQ in the development of opioid tolerance is also controversial. While one report showed attenuation of morphine tolerance after co-administration of the natural peptide ligand N/OFQ (Lutfy *et al.*, 2001), numerous studies suggested N/OFQ antagonists are useful as a co-treatment to prevent the development of morphine tolerance (Ueda *et al.*, 2000; Chung *et al.*, 2006). Consistent with its function as an 'anti-opioid' agent, endogenous

Table 1

Clinically reported analgesic effects and side effects mediated by each member of the opioid receptor family

		μ receptor	κ receptor	δ receptor	NOP receptor
Analgesic effects	Acute pain	+++	+	+	−
	Chronic pain	++	+	+++	++
	Neuropathic pain	+	+	+	+
	Inflammatory pain	+	+	+++	++
	Migraine	+	n.a.	+	n.a.
	Visceral pain	n.a.	+	n.a.	+
	Anti-allodynic	+	+	−/+	+
	Anti-depressant	+	+	+	−
Side effects	Rewarding	+	−	+	−
	Respiratory depression, cough reflex	+++	−	+	−
	Constipation, gastric motility	+++	+	+	−
	Euphoria	+	n.a.	n.a.	n.a.
	Dysphoria	n.a.	+	n.a.	n.a.
	Epilepsy	+	−	+	−
	Nausea, vomiting tolerance	+	−	−	−
	Dependence	+++	−	+	−
	Itch	+++	−	n.a.	−
	Renal function	+	+	+	+

The severity of the indicated effect is expressed as high (+++), moderate (++), low (+) or absent (−). No data available (n.a.).

N/OFQ may also be involved in precipitating side effects during opioid therapy (Zaveri, 2011). Due to the intimate functional interaction between the classical opioids and N/OFQ, recent attention has shifted to compounds that target both systems simultaneously.

This brief overview demonstrates that simultaneous targeting of multiple opioid receptors with compounds producing a mixture of agonistic and/or antagonistic effects may be a promising strategy to overcome the existing intrinsic limitations of the current opioid drugs.

Indeed, in the clinic, polypharmaceutical interventions are being employed to control opioid-mediated side effects. One strategy used to alleviate side effects and to lower the abuse potential of opioids is to combine the analgesically active opioid with an antagonist that reduces constipation. This concept, known as PAMORA (peripherally acting μ receptor antagonist) is already clinically established in a fixed-dose combination scheme of **buprenorphine** and **naloxone** (Suboxone™) or **oxycodone** and naloxone (Targin™). However, in this case the management of one side effect requires intervention with a second drug, which alleviates only one symptomatic condition, that is, constipation, while other centrally mediated side effects persist. Thus, the aim for development of multi-opioid receptor ligands should be a single chemical entity with improved analgesic activity and reduced side effects.

At first glance, the concept of addressing multiple opioid receptors seems rather old fashioned. Since the 1950s, a plethora of opioid compounds have been developed even though their pharmacology had not been elucidated due to a lack of molecular tools. Before the four members of the opioid receptor family were cloned in the early 1990s, the rationale in drug design was the creation of ever-more selective ligands, commonly based on natural alkaloids such as morphine, and optimization of their pharmacokinetic profile. When pure opioid receptor preparations in cells transfected with cloned receptor cDNAs became available, reexamination of opioid agonists revealed mixed pharmacological profiles of several clinically successful drugs, such as buprenorphine, **tramadol** and naltrexamine (Sadee *et al.*, 1982; Frink *et al.*, 1996; Cami-Kobeci *et al.*, 2009) Together with earlier *in vivo* work, it became clear that one alternative strategy for developing more effective analgesics could be to use either mixtures of selective opioid receptor agonists or single compounds with mixed pharmacological profiles, in order to produce synergistic analgesic effects, combined with hopefully fewer side effects. Recent progress in medicinal chemistry has suggested the possible design, synthesis and testing of single molecule entities with mixed opioid receptor agonist or combined agonist/antagonist activities.

What options are there to address opioid receptors?

All marketed opioids are orthosteric agonists with different intrinsic activity (full, partial or inverse agonists, or neutral antagonists) that target either one or multiple opioid receptors. Clinically used opioids such as morphine require the expression of μ receptors to exert an effect, as shown in μ receptor knock out mice (Matthes *et al.*, 1996). In contrast to orthosteric ligands, the design of allosteric modulators could help to control spatial and temporal receptor signalling in the presence or absence of the endogenous peptide opioid

ligands. While less conserved than orthosteric sites, the allosteric agonist-binding sites, when targeted, could produce higher ligand selectivity and therefore fewer off-target effects. A proof-of-concept approach generated positive allosteric modulators for the μ receptor, namely **BMS-986122**/**BMS-986121** (Burford *et al.*, 2013), and BMS-986187 for the δ receptor (Burford *et al.*, 2015), as shown in Figure 1. Another approach used to overcome the limited efficacy of classical orthosteric ligands is the development of compounds with biased signalling towards G-proteins or β-arrestins. The μ receptor agonist **TRV130** was the first example of a G-protein-biased substance to mediate analgesia with fewer side effects compared to morphine (DeWire *et al.*, 2013). The concept of 'biased' μ receptor agonists was recently extended by **PZM21**, which shows an even higher selectivity for G_i-activation and a favourable dissociation of analgesia from side effects in rodent models (Manglik *et al.*, 2016). The complex pharmacology of opioids, contrasted by the very limited number of opioid receptors, might be explained by the existence of receptor subtypes and the formation of heterodimers. Targeting heterodimerized opioid receptors has led to the recent development of CYM51010, a μ-δ receptor-heteromer-biased agonist (Gomes *et al.*, 2013). MDAN-21 was designed as a spacer-linked bivalent (heterobivalent) compound with an **oxymorphone**-derived μ receptor-binding moiety and **naltrindole**-related δ receptor-binding component, which suppressed morphine-dependent withdrawal signs in rhesus monkeys (Aceto *et al.*, 2012). These findings suggest the simultaneous occupancy of δ receptors with an antagonist and μ receptors with an agonist as a promising strategy to dissociate antinociception from side effects. While there is no bitopic (dualsteric) compound so far for opioid receptors, this has been made for other class A GPCRs, for example, the **M_1 muscarinic receptor** agonist **McN-A-343** (Valant *et al.*, 2008) or the dopamine **D_2 receptor** agonist **SB269652** (Mistry *et al.*, 2015).

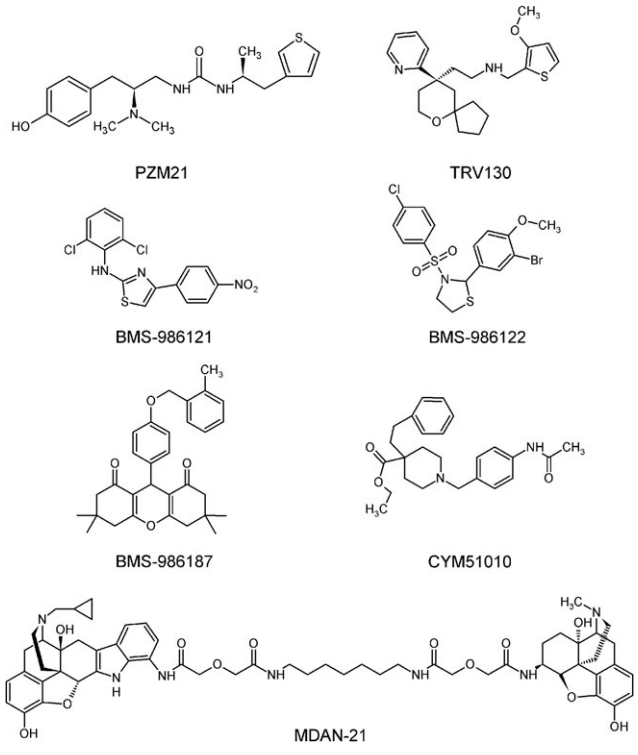

Figure 1
Structures of proof-of-concept opioid compounds.

Rationale for the development of multi-targeting opioid ligands

Structure and design of multi-targeting opioid ligands

In principle, the simultaneous activation of two receptors can be achieved with two different ligands, each binding the receptor with its cognate pharmacophore. In contrast to this approach, which requires two drugs, a more refined way is the design of a single compound containing the pharmacophores for activation of both receptors, thus designing ligands with a more predictable pharmacokinetic and pharmacodynamic profile (Morphy and Rankovic, 2009). The goal of targeting multiple opioid receptors with a single chemical entity can be realized by designing either bivalent or bifunctional (monovalent) compounds. While for bivalent ligands, two distinct pharmacophores have to be fused with a linker (Portoghese, 1989), bifunctional ligands are non-selective compounds that combine two or more receptor-specific binding properties (Li *et al.*, 2007). The recent development of multi-opioid receptor ligands is mainly centred on the development of bifunctional and bivalent ligands simultaneously addressing μ/NOP receptor or μ/δ receptor as shown in Figure 2.

μ/κ receptor mixed ligands

κ receptor agonists alone produce potent analgesia, especially in visceral pain models, but are often accompanied by many unwanted effects such as dysphoria and diuresis. However, they were found to reduce the rewarding effects of a variety of addictive drugs (Shippenberg *et al.*, 2007) and are, therefore, potential analgesics to overcome opioid dependence. The recent development of selective κ receptor agonists has focused on selective peripherally acting analgesics due to their dysphoric central effects. For example, the co-application of **fentanyl** with the κ receptor agonist **spiradoline** potentiates antinociception in rats (Briggs *et al.*, 1998). In addition to the μ receptor, the κ receptor is also expressed in the spinal cord, dorsal root ganglia (DRGs), peripheral sensory neurons and supraspinal regions like the rostral ventral medulla (RVM) or the periaqueductal grey (PAG). The evidence for whether μ and κ receptors form heterodimers is ambivalent (Jordan and Devi, 1999; Wang *et al.*, 2005). When applied individually, κ receptor agonists mediate antinociceptive effects. However, μ receptor activation can be partially attenuated by administration of the κ receptor agonist **U69593** (Ackley *et al.*, 2001), suggesting the existence of heterodimers *in vivo*, but not excluding a functionally antagonistic circuitry. The development of monofunctional κ receptor agonists is focused on the concept of targeting κ receptors in the periphery, with an emphasis on managing visceral pain (Black and Trevethick, 1998). The κ receptor agonist **nalfurafine** is used clinically in Japan as an antipruritic

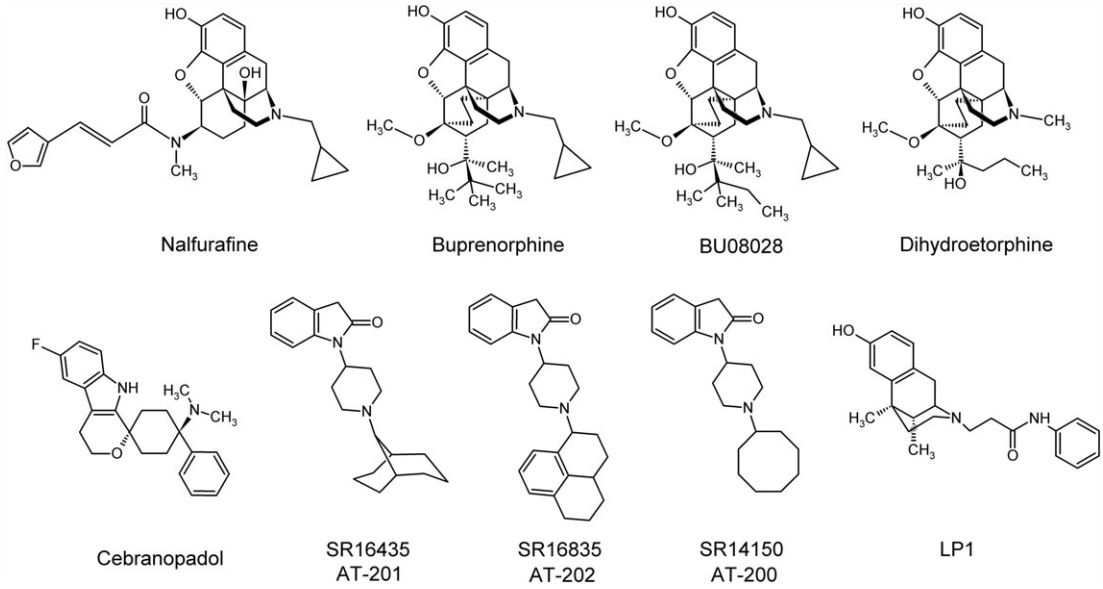

Figure 2

Structures of multi-opioid receptor ligands.

for the treatment of intractable pruritus in haemodialysis patients (Kumagai *et al.*, 2010; Nakao *et al.*, 2016) and also exhibits high-antinociceptive activities (Endoh *et al.*, 1999, 2000). Receptor binding and functional studies of recombinant human and rat opioid receptors have shown nalfurafine to be a potent and selective agonist at the κ receptor, but also to display weak and partial agonist activity at μ and δ receptors (Seki *et al.*, 1999). μ receptor agonists carry a high risk of abuse potential, whereas selective κ receptor agonists generally lack a reinforcing effect (Negus *et al.*, 2008). Accordingly, nalfurafine produced no reinforcing effects in rhesus monkeys in an intravenous self-administration paradigm. These data indicated that nalfurafine can be used for long-term treatment of intractable pruritus with minimal concern regarding the risk of dependence (Nakao *et al.*, 2016). All other mixed μ/κ receptor agonists in clinical use, such as **butorphanol**, **nalbuphine** and **pentazocine**, alleviate morphine-induced pruritus; however, only butorphanol is used to treat chronic pruritus (Du *et al.*, 2013). Dihydroetorphine is one of the most potent analgesic opioids known, with up to 12-fold higher potency than morphine. Several *in vitro* and *in vivo* studies have shown that dihydroetorphine is a μ receptor agonist that also binds and activates κ and δ receptors (Katsumata *et al.*, 1995). The onset of its analgesic action is rapid, its duration of action after systemic administration to rodents is rather short and it is largely ineffective after oral administration (Ohmori *et al.*, 2000; Ohmori and Morimoto, 2002). Continuous exposure and repeated administration of dihydroetorphine results in analgesic tolerance, physical dependence, and a rewarding effect in normal animals, but not in rats with formalin-induced inflammation (Ohmori *et al.*, 2000). Clinical tests in China using sublingual application of dihydroetorphine showed a potent analgesic effect with only mild side effects, which included dizziness, somnolence, nausea, vomiting, constipation and shortness of breath (Ohmori *et al.*, 2000; Ohmori and Morimoto, 2002). Transdermal delivery of dihydroetorphine via a patch produces continuous analgesic effects with minimal physical dependence and rewarding effects in rats suffering from chronic pain (Ohmori *et al.*, 2000). While κ receptor agonists frequently precipitate dysphoric mood changes in humans and depression-like symptoms in experimental animals (Ranganathan *et al.*, 2012), several κ receptor antagonists were found to exert anti-depressant effects (Lutz and Kieffer, 2013) (Table 2). A combined pharmacological profile of a μ receptor agonist and κ receptor antagonist could thus not only produce analgesia but also treat depressive symptoms, a frequent comorbidity in chronic pain patients (Taylor and Manzella, 2016). The compound best studied in this context is buprenorphine, which is a partial μ receptor agonist with a weak κ receptor antagonistic profile. Buprenorphine was found to produce antidepressant and anxiolytic effects in mice, which were mediated through the κ receptor (Falcon *et al.*, 2015), although clinical observations in chronic pain patients treated with buprenorphine failed to detect lasting mood effects (Stein *et al.*, 2015). Overall, compounds simultaneously targeting μ and κ receptors, either as agonist/agonist or agonist/antagonist, have shown some clinical efficacy, although the main limitations of chronic μ receptor activation, such as abuse liability, constipation and respiratory depression, are still apparent.

μ/δ receptor mixed ligands

The main rationale behind simultaneously targeting μ/δ receptors is to develop drugs with attenuated tolerance profiles suitable for the management of persistent pain conditions (Abdelhamid *et al.*, 1991). The concomitant application of the δ receptor agonist **SNC80** with a μ receptor agonist such as fentanyl potentiated the antinociceptive actions of the δ receptor agonist (Stevenson *et al.*, 2003). The mixed μ/δ receptor antagonist LP1 was reported as a

Table 2

Activity profile of selected multi-opioid receptor ligands

Substance	μ receptor	κ receptor	δ receptor	NOP receptor	Profile/activity	Source
Dihydroetorphine	+++	+++	+++	n.a./−	analgesic for severe and cancer pain	Ohmori *et al.* 2000, Ohmori and Morimoto, 2002, Ranganathan *et al.*, 2012
Nalfurafine	+++	+++	+	+	antipruritic and antinociceptive activity; clinical trial for pruritus	Kumagai *et al.*, 2010, Nakao *et al.*, 2016, Endoh *et al.*, 1999
Buprenorphine	++	n.a/−	n.a/−	+	opioid substitution therapy; postoperative pain control; chronic pain; cancer pain; neuropathic pain in combination with full μ receptor agonists	Lutfy and Cowan, 2004, Huang *et al.*, 2001, Khroyan *et al.*, 2009a, 2009b, van Niel *et al.*, 2016, Bonhomme *et al.*, 2012
BU08028	+++	−	+	++	antinociceptive, antihypersensitive and antiallodynic activity; absence of respiratory and cardiovascular activities; absence of physical dependence and pruritus	Ding *et al.*, 2016, Khroyan *et al.*, 2011
SR14150 (AT-200)	++	+	n.a.	+++	antinociceptive	Spagnolo *et al.* 2008, Khroyan *et al.*, 2007, Toll *et al.*, 2009, Sukhtankar *et al.*, 2013
SR16435 (AT-201)	+++	+	−	++	antinociceptive and antiallodynic, induces CPP, slower development of tolerance compared to μ receptor agonists under neuropathic pain	
SR16835 (AT-202)	++	+	n.a.	+++	not antinociceptive, induces no CPP, attenuates morphine-mediated CPP	
Cebranopadol	+++	++	+	+++	clinical trial for severe chronic and neuropathic pain	Linz *et al.* 2014

The grade of intrinsic activity is expressed as high (+++), moderate (++), low (+) or absense (−) of intrinsic activity determined by GTPγS assay. (n.a.) Data are either not available or were not determined.

valuable substance for the treatment of persistent pain conditions (Parenti *et al.*, 2013). Compared to the pain relief triggered upon μ receptor activation in acute pain states, activation of the δ receptor alone is relatively ineffective (Gallantine and Meert, 2005). However, δ receptor activation can be therapeutically beneficial in the treatment of chronic pain states, especially inflammatory pain (Gaveriaux-Ruff *et al.*, 2008), and co-administration of a δ receptor agonist with a μ receptor agonist increases the overall analgesic effects (Kovelowski *et al.*, 1999). There is less abuse potential for δ receptor agonists compared to μ receptor agonists (Carmo *et al.*, 2009), fewer gastro intestinal-related problems (Tavani *et al.*, 1990) and less respiratory depression (Szeto *et al.*, 1999). Another reason for simultaneously targeting μ and δ receptors is their co-localization in nociceptive sensory neurons (Wang *et al.*, 2010) and receptor crosstalk (Zhang and Pan, 2010). The existence of μ/δ receptor heterodimers with a unique binding pocket, signalling and trafficking (Gomes *et al.*, 2004) has led to the development of bivalent ligands. In contrast to other opioid receptors, the δ receptor was mainly found to be located intracellularly in basal conditions. Different stimuli, such as stress (Commons, 2003), chronic morphine exposure (Hack *et al.*, 2005), neuropathic and inflammatory pain (Cahill *et al.*, 2003; Kabli and Cahill, 2007) were demonstrated to enhance δ receptor-mediated analgesia as a function of membrane trafficking. In a proof-of-concept study for a mixed μ/δ receptor agonist, a bifunctional enkephalin-like peptide exhibited antiallodynic and antihyperalgesic effects in rats (Lee *et al.*, 2011). Conjointly targeting μ and δ receptors is a therapeutic option to potentially lower opioid tolerance in neuropathic pain, compared to classical opioids (Balboni *et al.*, 2010). As a general observation from these studies, it appears that δ receptor agonists require μ receptors for their activity. A special incentive for the development of dual μ/δ receptor agonists is the anti-depressant like activity induced by δ receptor agonists. Based on this concept, the fentanyl-like dual μ/δ receptor agonist RV-Jim-C3 was designed, which acts as an effective antinociceptive compound in neuropathic pain models with limited motor-activating effects (Podolsky *et al.*, 2013). The benzomorphan-based LP1 was found to have potent supraspinal antinociceptive activity in the tail flick test with longer lasting antinociception compared to morphine (Parenti *et al.*, 2012). However, further studies on the possible mood-elevating properties of these compounds have not yet been published. The evaluation of a novel class of 6β-acylaminomorphinans revealed that 6β-cinnamoylmorphinamine is a potent analgesic and that its effects can be attenuated by selective antagonists of μ and δ receptors. Interestingly, no alterations in respiratory rate were found in mice (Varadi *et al.*, 2013).

μ/NOP receptor mixed ligands

The μ and NOP receptor share common signalling pathways and are both functionally expressed in pain pathways such as the spinal cord, PAG and RVM, although mostly in distinctly separate neurons. There is evidence of only limited co-localization of μ and NOP receptors in DRGs as recently shown in NOP receptor-eGFP knock-in mice (Ozawa *et al.*, 2015). Also the heterodimerization of μ and NOP receptors, as reported in heterologous expression systems, is still controversial (Pan *et al.*, 2002). Probably most important for drug development was the discovery that NOP receptor agonists show better potential for the treatment of neuropathic pain than classical opioids (Schroder *et al.*, 2014). While the results from rodent studies produced conflicting views on the role of N/OFQ and the NOP receptor in analgesia, in rhesus monkey studies the NOP receptor has been shown to be a valid target for the treatment of pain (Ko *et al.*, 2009b). These effects appear to be mediated at the spinal level and peripheral nociceptive sensory neurons. In addition, early studies indicated the potential of mixed μ/NOP receptor agonists to alleviate some of the side effects of pure μ receptor agonists, including the development of tolerance and dependence. Counterintuitively, a potential mechanism for this desirable effect might be chronic desensitization of NOP receptor signalling in reward and tolerance circuits (Lutfy *et al.*, 2001), since similar effects were also reported for a co-treatment regimen with μ receptor agonists and NOP receptor antagonists (Ueda *et al.*, 2000; Chung *et al.*, 2006). Based on these findings, a clear rationale for the design of bivalent μ/NOP receptor agonists was provided (Zaveri *et al.*, 2013), which was supported by observations from opioid compounds that are already in clinical use, such as buprenorphine. Buprenorphine, is a unique opioid compound with mixed agonist–antagonist activity at classical opioid receptors that has been approved for the treatment of opioid dependency and is also used as an analgesic (Lutfy and Cowan, 2004). Buprenorphine has partial intrinsic agonistic activities at μ and NOP receptors and very low intrinsic activity at κ and δ receptors (Huang *et al.*, 2001). The buprenorphine dose–response curve is sometimes submaximal, or even bell-shaped, in nociceptive assays (Lutfy and Cowan, 2004). Partial agonism at μ receptors and, in some cases, antagonism at κ or δ receptors have been considered as possible underlying mechanisms for the ceiling effect and bell-shaped dose–response curve of buprenorphine. While ceiling effects can be explained by the partial agonist activity of buprenorphine, the bell-shaped dose–response curve has been postulated to be due to the suppression of μ receptor efficacy at higher doses by its NOP receptor partial agonist activity, at least in acute pain assays (Khroyan *et al.*, 2009a). Buprenorphine's analgesic activity has been characterized in several acute and chronic pain models and it is a very potent long-lasting analgesic that is useful after surgery in humans (Khroyan *et al.*, 2009a). In the clinical setting, analgesia induced by buprenorphine is indiscernible from that of a full μ receptor agonist, without a ceiling effect (van Niel *et al.*, 2016). However, there is a ceiling effect for respiratory depression, reducing the likelihood of this potentially fatal adverse event occurring (Dahan *et al.*, 2006). The ceiling effect may also be responsible for the well-known reduced abuse liability of buprenorphine, which is therefore often used as a substitute opioid in heroin replacement programmes (Bonhomme *et al.*, 2012). Overall, these properties make it useful for opioid maintenance therapy (Khroyan *et al.*, 2009a; Mello and Mendelson, 1980). It is also reported that buprenorphine does not diminish morphine-induced antinociception, and even interacts in an additive to supra-additive manner with morphine in preclinical rodent models (van Niel *et al.*, 2016).

The buprenorphine analogue **BU08028** with mixed μ/NOP receptor agonist activity was designed to have increased affinity and efficacy at NOP receptors. BU08028 is the first reported 'universal opioid ligand' that binds with high affinity to each member of the opioid receptor family, with a higher functional efficacy at the NOP receptor compared with buprenorphine *in vitro* (Ding *et al.*, 2016; Khroyan *et al.*, 2011). BU08028 produces long-lasting antinociceptive and antihypersensitive actions mediated by both receptors, without reinforcing effects. In contrast to other μ receptor agonists, BU08028 possesses a higher safety profile and does not either inhibit respiratory functions, impair cardiovascular activities or produce acute physical dependence (Ding *et al.*, 2016). In recent years, a series of modestly selective small-molecule NOP receptor agonists has been developed, such as SR14150 (AT-200) and **SR16835** (AT-202), and a non-selective μ/NOP receptor agonist SR16435 (AT-201). These bifunctional ligands, which can simultaneously activate both NOP and μ receptors, have a wider therapeutic window and the ability to alleviate severe pain conditions. Both SR14150 and SR16835 have higher selectivity and efficacy at NOP receptors compared to SR16435, and lower affinity and efficacy at μ receptors (Toll *et al.*, 2009). SR14150 has antinociceptive activity in the tail-flick assay that seems to be caused by activation of the μ receptor, because it was naloxone-reversible. This suggests that the partial agonist efficacy of SR14150 at the μ receptor is sufficient to produce an acute antinociceptive effect without rewarding effects. SR16835 has slightly lower NOP receptor affinity than SR14150 and does not produce μ receptor-mediated behavioural effects, such as conditioned place preference (CPP) (Toll *et al.*, 2009). In a chronic pain model (sciatic nerve ligation in mice), both SR14150 and SR16835 display potent antiallodynic activity, with SR14150 being more potent than SR16835. In contrast to thermal nociception, this effect was completely blocked by the NOP receptor antagonist **SB 612111** but was not altered by naloxone, indicating that both compounds have NOP receptor-mediated and not μ receptor-mediated antiallodynic activity (Khroyan *et al.*, 2011). In contrast, SR16435, a partial agonist at μ and NOP receptors, is a potent analgesic with reduced and slower development of tolerance to its antiallodynic effects compared to buprenorphine, but produces CPP to the same extent as morphine (Khroyan *et al.*, 2007; Sukhtankar *et al.*, 2013). The NOP receptor antagonist SR14148 was ineffective on its own but found to potentiate the antiallodynic activity of morphine and the mixed NOP/μ receptor agonist SR16435. Therefore, NOP receptor antagonists may provide a favourable therapeutic combination for neuropathic pain treatment that would allow for a reduction in opioid dosage. Furthermore, modulation of μ receptor-mediated analgesia by NOP receptors using dual ligands with a profile of MOR agonist

and NOPR antagonist activity may be particularly useful for the treatment of chronic pain (Khroyan *et al.*, 2009b). In contrast, NOP receptor mediated antiallodynic activity may be more effective than that induced by an opiate medication and both NOP receptor agonists and antagonists could potentially become useful treatments for neuropathic pain (Khroyan *et al.*, 2011). Importantly, compounds with affinity for both NOP and μ receptors may have a useful profile as analgesics with slower tolerance development and reduced addiction liability (Toll *et al.*, 2009; Cremeans *et al.*, 2012). **Cebranopadol**, a novel mixed μ/NOP receptor ligand, is currently in clinical development (phase 3 clinical trial) for the treatment of severe chronic nociceptive and neuropathic pain that targets the NOP receptor and other members of the opioid receptor family, in particular the μ receptor (Linz *et al.*, 2014). Cebranopadol has full agonist activity at μ receptors, near-full activity at the κ and NOP receptor, and partial activity at δ receptors. In various pain states, cebranopadol displays broad activity and is highly potent and efficacious in animal models of acute nociceptive, inflammatory, cancer and, especially, chronic neuropathic pain. Furthermore, even when administered at doses higher than those required to produce analgesia, cebranopadol does not affect either motor coordination or respiratory function and thus displays a better tolerability profile than opioids. In contrast to morphine, cebranopadol displays higher analgesic potency in chronic pain, especially of neuropathic origin, than in acute nociceptive pain (Linz *et al.*, 2014). Both BU08028 and cebranopadol are proof-of-concept compounds to show that ligands with a multi-opioid receptor pharmacology can produce a clinically useful therapeutic spectrum with a reduced side effect profile.

Conclusions

The search for improved opioid analgesics has clearly changed direction during the past two decades. When the first molecular structures of the four members of the opioid receptor family became available in the early 1990s, the initial hope was that ever-more selective compounds targeting a single opioid receptor may eventually lead to more potent analgesics with fewer or no side effects. Subsequently, a deeper understanding of the anatomy, physiological functions and interactions between the four opioid systems, and last but not least detailed insights into the molecular basis of distinct intracellular signal transduction pathways of all members of the opioid receptor family have changed this view. Consequently, compounds that were until recently denounced as 'dirty drugs', because they interacted with multiple opioid receptors, now receive new attention. New research has demonstrated the value of simultaneously targeting two or even more members of the opioid receptor family, thus applying these new insights into the complex functional interactions of these pain-modulating systems for devising new strategies in drug development.

The goal has always remained the same: creating a 'better morphine', with analgesic effects equal to morphine but none of the very serious liabilities, such as dependence, or possibility of fatal overdoses. Most progress has been made in the development of μ/NOP receptor bifunctional agonists for which the scientific rationale is probably strongest at this moment. But similar cases can be made for μ/δ receptor and μ/κ receptor mixed compounds, especially when taking into account a more narrow therapeutic application for specific pain states. The mixed μ/κ receptor agonist nalfurafine and its successful development for treatment of pruritus is a good example for the therapeutic benefits of such novel opioids with a mixed pharmacological profile that are highly effective in a limited therapeutic spectrum. The two μ/NOP receptor mixed agonists BU08028 and cebranopadol independently showed their potential for treatment of chronic inflammatory and neuropathic pain, which are largely unmet therapeutic needs.

It appears that balancing efficacies at multiple opioid receptors with a single compound may be a promising avenue to develop new opioid analgesics that are able to address specific pain conditions, which are not adequately managed with the drugs currently available. This strategy is not in competition, but hopefully complementary, to the recently developed concept of biased μ receptor agonists, both aim to reveal novel opioid analgesics with improved pharmacological profiles.

Outstanding issues

Do opioid receptors have to be compartmentalized when they are addressed with one ligand?

Is targeting multi-opioid receptors one step back in the emerging search for selective ligands?

Is the peripheral restriction of the targeted opioid receptors the key to overcome opioid-mediated side effects?

What is the perfect balance of ligands with a preferential μ/NOP receptor profile?

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.*, 2016), and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 (Alexander *et al.*, 2015).

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Conflict of interest

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